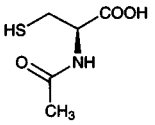
Acetylcysteine



Molecular formula: $C_5H_9NO_3S$
Molecular weight: 163.20
CAS Registry No.: 616-91-1
Merck Index: 89

SAMPLE

Matrix: blood

Sample preparation: Add 90 μL cold 10% trichloroacetic acid (containing 1 mM disodium EDTA) to 10 μL blood. Centrifuge at 1850 g at 0° for 5 min. Dilute a 10 μL aliquot of the supernatant with 2 mL water. Mix a 200 μL aliquot of the supernatant with 100 μL 100 μM o-phthalaldehyde reagent and 100 μL 200 μM N-(4-aminobutyl)-N-ethylisoluminol reagent, vortex thoroughly. Let stand for about 2 min. Inject a 20 μL aliquot of the reaction mixture. (Prepare reagents as follows. Dissolve o-phthalaldehyde in 50 mM pH 9.0 sodium borate buffer containing 100 mM potassium dihydrogen phosphate to give a 100 μM solution. Prepare a 1 mM solution of N-(4-aminobutyl)-N-ethylisoluminol (chemiluminescence-grade, Tokyo Kasei) in MeOH containing 5 mM HCl. Dilute with MeOH to give a 200 μM solution.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrosorb RP-18
Column: 150 × 4.6 5 μm Cosmosil 5C18-AR (Nacalai Tesque)
Mobile phase: MeOH:THF:100 mM pH 7.5 phosphate buffer 25:5:70
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence, TOA Electronics ICA-3070 detector following post-column reaction. The column effluent mixed with 150 mM hydrogen peroxide pumped at 0.2 mL/min and 25 μM hematin in 150 mM sodium carbonate buffer pumped at 3 mL/min and this mixture flowed through a 200 × 0.5 mm PTFE reaction coil to the detector.

CHROMATOGRAM

Retention time: k' 3.1
Limit of detection: 3.5 nM

OTHER SUBSTANCES

Extracted: captopril

KEY WORDS

post-column reaction; derivatization

REFERENCE

Sano,A.; Nakamura,H. Chemiluminescence detection of thiols by high-performance liquid chromatography using o-phthalaldehyde and N-(4-aminobutyl)-N-ethylisoluminol as precolumn derivatization reagents, *Anal.Sci.*, **1998**, *14*, 731–735.

SAMPLE

Matrix: blood

Sample preparation: Vortex 750 μL red blood cells with 3.2 mL 100 mM HCl, centrifuge at 4° at 15000 rpm for 5 min. Remove the supernatant and mix it with an equal volume of cold 4 M sodium methanesulfonate, freeze in dry ice/isopropanol, thaw, centrifuge at 20000 rpm for 10 min. Determine the thiol content of the supernatant by titrating with 5,5'-dithiobis(2-nitrobenzoic acid). Add N-ethylmorpholine to a final concentration of 10 mM, adjust pH to 8.0 with 1 M NaOH, add a 1 molar equivalent of dithiothreitol, mix, let stand at room temperature for 5 min, add 6 equivalents of monobromotrimethylam-

moniobimane, mix, let stand for 15 min, add 12 equivalents of thiol agarose, let stand for 20 min, add acetic acid to a final concentration of 3%, inject an aliquot. (Prepare 4 M sodium methanesulfonate by adjusting the pH of methanesulfonic acid to 1.5 with 50% NaOH then diluting to 4 M.)

HPLC VARIABLES

Column: 150 × 4 AA-10 resin (Beckman)

Mobile phase: Gradient. A was 2-methoxyethanol:200 mM pH 3.20 buffer 10:90 at 45°. B was 2-methoxyethanol:200 mM pH 4.40 buffer 10:90 at 45°. C was 2-methoxyethanol:200 mM pH 4.75 buffer 10:90 at 45°. D was 2-methoxyethanol:0.2 N pH 6.40 trisodium citrate containing 800 mM NaCl 10:90 at 55°. E was 2-methoxyethanol:100 mM NaOH containing 100 mM NaCl 10:90 at 55°. A for 10 min; B for 20 min; C for 5 min, D for 90 min, E for 10 min, re-equilibrate at initial conditions for 20 min.

Flow rate: 0.2

Detector: F (o-phthalaldehyde filters)

CHROMATOGRAM

Retention time: 27.5

Limit of detection: 1 pmole

Limit of quantitation: 10 pmole

OTHER SUBSTANCES

Extracted: coenzyme A, coenzyme M, cysteine, cysteinylglycine, dithiothreitol, ergothioneine, gamma-glutamylcysteine, glutathione, homocysteine, 2-mercaptoethanol, methanethiol, pantetheine, 4'-phosphopantetheine, thiosulfate, thiouracil

KEY WORDS

derivatization; red blood cells

REFERENCE

Fahey,R.C.; Newton,G.L.; Dorian,R.; Kosower,E.M. Analysis of biological thiols: Quantitative determination of thiols at the picomole level based upon derivatization with monobromobimanes and separation by cation-exchange chromatography, *Anal.Biochem.*, **1981**, *111*, 357–365.

SAMPLE

Matrix: blood

Sample preparation: Vortex 750 μL red blood cells with 3.2 mL 100 mM HCl, centrifuge at 4° at 15000 rpm for 5 min. Remove the supernatant and mix it with an equal volume of cold 4 M sodium methanesulfonate, freeze in dry ice/isopropanol, thaw, centrifuge at 20000 rpm for 10 min. Determine the thiol content of the supernatant by titrating with 5,5'-dithiobis(2-nitrobenzoic acid). Add N-ethylmorpholine to a final concentration of 10 mM, adjust pH to 8.0 with 1 M NaOH, add a 1 molar equivalent of dithiothreitol, mix, let stand at room temperature for 5 min, add 6 equivalents of monobromobimane, mix, let stand for 15 min, add 12 equivalents of thiol agarose, let stand for 20 min, add acetic acid to a final concentration of 3% (Anal. Biochem. 1981, 111, 357), dilute with 200 mM pH 2.2 sodium citrate, inject an aliquot. (Prepare 4 M sodium methanesulfonate by adjusting the pH of methanesulfonic acid to 1.5 with 50% NaOH then diluting to 4 M.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere-ODS C18

Mobile phase: Gradient. A was MeOH:water:acetic acid 10:89.75:0.25, adjusted to pH 3.9 with 50% NaOH. B was MeOH:water:acetic acid 90:9.75:0.25, adjusted to pH 3.9 with 50% NaOH. A:B 92:8 for 10 min, to 60:40 over 10 min, maintain at 60:40 for 5 min, to 10:90 over 5 min, to 0:100 over 2 min.

Flow rate: 1.5

Detector: F (o-phthalaldehyde filters)

CHROMATOGRAM
Retention time: 11.8
Limit of detection: 2-20 pmole

OTHER SUBSTANCES
Extracted: coenzyme A, coenzyme M, cysteamine, cysteine, cysteinylglycine, ergothioneine, ethanethiol, gamma-glutamylcysteine, glutathione, homocysteine, hydrogen sulfide, 2-mercaptoethanol, mercaptopyrimidine, methanethiol, pantetheine, 4'-phosphopantetheine, thiosulfate, 2-thiouracil

KEY WORDS
derivatization; red blood cells

REFERENCE
Newton,G.L.; Dorian,R.; Fahey,R.C. Analysis of biological thiols: derivatization with monobromobimane and separation by reverse-phase high-performance liquid chromatography, *Anal.Biochem.*, **1981**, *114*, 383–387.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 200 μL 200 g/L trichloroacetic acid, vortex, let stand for 15 min, centrifuge. Remove 600 μL supernatant and add it to 400 μL 20 mM disodium EDTA and 1 mL 240 mM pH 10.0 borate buffer, add 1 mL of Thiopropyl-Sepharose 6B (containing 20 μmol thiol per mL of gel suspension) (Pharmacia), place in an end-over-end mixer for 30 min, add 1 mL 4 M acetic acid, centrifuge. Add 3 mL of the supernatant to a 13 × 7 column of p-acetoxymercurianiline-Sepharose 4B (Biochim.Biophys.Acta 1970, 200,593), wash with two 1 mL portions of water, elute with 3 mL 10 mM cysteine hydrochloride. Add the eluate followed by 1 mL 10 mM HCl to a 25 × 5 AG 50W-X8 (H+) 100-200 mesh column (Bio-Rad). Collect the effluents and add them to 200 μL 180 mM disodium EDTA. Remove a 2 mL aliquot and add it to 200 μL 100 mM NaOH, add 3 mL 50 mM pH 9.0 carbonate buffer containing 10 mM disodium EDTA, add 500 μL 20 μM N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide in acetone, heat at 37° overnight, dilute 1:5 with mobile phase, inject a 100 μL aliquot onto column A. When the acetylcysteine has eluted from column A to column B remove column A from the circuit, monitor the effluent from column B. Backflush column A with mobile phase to clean it.

HPLC VARIABLES
Column: A 30 × 4.6 10 μm 10 μm Brownlee RP-8; B 250 × 4.6 5 μm Supelcosil LC-8
Mobile phase: MeOH:2 mM sodium phosphate 15:85, containing 10 mM tetramethylammonium hydroxide, pH adjusted to 7.4 with 6 M HCl
Flow rate: 0.75
Injection volume: 100
Detector: F ex 360 (filter) em 418-700 (filter)

CHROMATOGRAM
Retention time: 15

KEY WORDS
plasma; column-switching; SPE

REFERENCE
Kagedal,B.; K:allberg,M.; Martensson,J. Determination of non-protein-bound N-acetylcysteine in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *311*, 170–175.

SAMPLE
Matrix: blood

Sample preparation: 100 μL Plasma + 5 μL 100 mM dithiothreitol in 10% Triton X-100, vortex, allow to stand for 30 min, add 100 μL 30 mM monobromobimane (Calbiochem; Molecular Probes) in 50 mM pH 8.0 N-ethylmorpholine, store in the dark for 5 min, add 10 μL 100% (w/v) trichloroacetic acid, centrifuge at 3000 g for 3 min, inject a 25 μL aliquot of the supernatant. (Dissolve monobromobimane in the minimum amount of MeCN before making up aqueous solutions.)(J. Biochem. Biophys. Methods 1986, 13, 231)

HPLC VARIABLES

Column: 75 × 4.5 3 μm Supelco octadecylsilica
Mobile phase: Gradient. A was MeCN:acetic acid:perchloric acid:water 9:0.25:0.25:90.75, pH 3.7. B was MeCN:water:perchloric acid 75:25:0.25. A:B 100:0 for 7 min then 0:100 for 4 min then re-equilbrate at 100:0 for 7 min.
Flow rate: 1
Injection volume: 25
Detector: F ex 394 em 480

CHROMATOGRAM

Retention time: 7.4
Limit of detection: 0.5 nM

KEY WORDS

plasma; derivatization

REFERENCE

Cotgreave,I.A.; Moldéus,P. Methodologies for the analysis of reduced and oxidized N-acetylcysteine in biological systems, *Biopharm.Drug Dispos.*, **1987**, *8*, 365–375.

SAMPLE

Matrix: blood, tissue
Sample preparation: Tissue. Homogenize (Potter-Elvehjem PTFE-glass homogenizer) tissue in 20 mM EDTA, adjust to 1% (w/v) (kidney) or 2.5% (w/v) (spleen). Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Plasma. Dilute rat plasma to 20% (v/v) with 20 mM EDTA. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. Serum. Dilute human serum to 10% (v/v) with 20 mM EDTA. 1 mL Diluted serum + 200 μL 30% metaphosphoric acid, centrifuge at 2000 g at 4° for 20 min. Remove 500 μL of the supernatant and add it to 240 μL 2 M KOH. Remove 100 μL of this solution and add it to 400 μL 100 mM pH 8.5 borate buffer, add 300 μL 0.24 mM N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide in MeCN, add 200 μL IS, heat at 60° for 30 min, cool for 5 min, centrifuge at 4° at 2000 g for 15 min, filter (Millipore 2 μm) the supernatant, inject a 20 μL aliquot of the supernatant. (Synthesis of N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide is as follows. Add 8.8 g aluminum trichloride to 12.50 g 3-dimethylaminophenol in 185 mL chloroform and 84 g triethyl orthoformate, mix at room temperature for 10 min, when the exothermic reaction ceases add 50 mL 10% HCl, stir to hydrolyze the acetal, neutralize with 10% NaOH, filter through a short column of Celite, wash through with chloroform, wash the filtrate with saturated aqueous NaCl, dry over magnesium sulfate, concentrate under reduced pressure, recrystallize from chloroform to give 4-(dimethylamino)salicylaldehyde (mp 78-79°). Add 400 mg KOH in 3 mL EtOH to a solution of 1 g 4-(dimethylamino)salicylaldehyde and 1.3 g (?) 4-nitrobenzylbromide in 12 mL EtOH, reflux for 7 h, cool, filter to recover the crystals, wash with water, dry under vacuum, recrystallize from EtOH to give 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde (mp 179-180°). Add a solution of 900 mg 4-dimethylamino-2-(4-nitrobenzyloxy)benzaldehyde in 6 mL DMF to a sodium methoxide

solution (prepared from 69 mg sodium in 1 mL MeOH), reflux for 20 min, add 1 mL MeOH, filter the crystals, recrystallize from EtOH to give 6-dimethylamino-2-(4-nitrophenyl)benzofuran as red needles (mp 209.5-210.5°). Reflux 1 g 6-dimethylamino-2-(4-nitrophenyl)benzofuran in 20 mL benzene (Caution! Benzene is a carcinogen!) and 18 mL MeOH containing 80 mg active carbon and a catalytic amount of ferric chloride hexahydrate for 10 min, add 2.30 g 98% hydrazine hydrate (Caution! Hydrazine hydrate is a carcinogen!) dropwise, reflux for 7 h, filter, concentrate the filtrate, recrystallize from cyclohexane to give 6-dimethylamino-2-(4-aminophenyl)benzofuran as orange needles (mp 198.5-200°). Stir 605 mg 6-dimethylamino-2-(4-aminophenyl)benzofuran and 230 mg maleic anhydride in 5 mL chloroform at room temperature for 3 h, filter the crystals, wash with a small amount of chloroform, recrystallize from EtOH to obtain N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid (mp 219.5-221°). Reflux a mixture of 1.17 g N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleamic acid and 30 mg sodium acetate in 18 mL acetic anhydride, cool in an ice bath, collect the crystals of product, wash with water. Neutralize the filtrate with 20% NaOH, extract twice with 30 mL portions of chloroform, wash the organic layers with saturated aqueous NaCl, dry over anhydrous magnesium sulfate, evaporate to give more product. Combine the products and recrystallize them from acetone to give N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]maleimide as reddish purple crystals (mp 203-204°) (Bull.Chem.Soc.Jpn. 1985, 58, 2192).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Toyo Soda ODS-80
Mobile phase: MeCN:10 mM pH 7.7 phosphate buffer 47.5:52.5 containing 30 mM tetrabutylammonium bromide
Flow rate: 0.8
Injection volume: 20
Detector: F ex 355 em 457

CHROMATOGRAM

Retention time: 6
Internal standard: disodium 6-amino-1,3-naphthalene disulfonate (3.5)
Limit of detection: 20 fmole

OTHER SUBSTANCES

Extracted: homocysteine, reduced glutathione (GSH), cysteamine, cysteine, coenzyme A

KEY WORDS

plasma; serum; rat; human; liver; kidney; spleen; derivatization

REFERENCE

Nakashima,K.; Umekawa,C.; Yoshida,H.; Nakatsuji,S.; Akiyama,S. High-performance liquid chromatography-fluorometry for the determination of thiols in biological samples using N-[4-(6-dimethylamino-2-benzofuranyl)phenyl]-maleimide, *J.Chromatogr.*, **1987**, *414*, 11–17.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition CF50A Centriflo ultrafiltration cones (Amicon) by immersing in water for 1 h and centrifuging at 1000 g for 5 min. 3 mL Plasma + 1 mL water + 500 μL 5 mg/mL dithiothreitol, vortex for 10 s, incubate at 37° for 30 min, add 1 mL 2% sodium bicarbonate, add 350 μL 5% 2,4-dinitro-1-fluorobenzene in EtOH, vortex for 10 s, incubate at 60° for 30 min, place in ultrafiltration cone, centrifuge at 1000 g at 20° for 20 min. Remove 1 mL of the ultrafiltrate and add it to 5 mL water and 10 mL ether, shake mechanically in the dark at 250 cycles/min for 5 min, centrifuge at 1000 g at 10° for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 75 μL mobile phase. Urine. 100 μL urine + 1 mL water + 500 μL 5 mg/mL dithiothreitol, vortex for 10 s, incubate at 37° for 30 min, add 1 mL 2% sodium bicarbonate, add 350 μL 5% 2,4-dinitro-1-fluorobenzene in EtOH, vortex for 10 s, incubate at 60° for 30 min. Remove 1 mL and add it to 5 mL water and 10 mL

ether, shake mechanically in the dark at 250 cycles/min for 5 min, centrifuge at 1000 g at 10° for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:50 mM trisodium citrate and 1 mM EDTA adjusted to pH 7.0 with citric acid solution 30:70 (plasma) or 35:65 (urine)
Flow rate: 1
Injection volume: 50
Detector: UV 360

CHROMATOGRAM
Retention time: 13
Limit of detection: 50 ng/mL

KEY WORDS
plasma; derivatization; ultrafiltration

REFERENCE
Lewis,P.A.; Woodward,A.J.; Maddock,J. High-performance liquid chromatographic assay for N-acetylcysteine in plasma and urine, *J.Pharm.Sci.*, **1984**, *73*, 996–998.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out tablets, capsule contents, or granules equivalent to 50 mg N-acetylcysteine, add 10 mL IS solution, add 80 mL 0.05% sodium hydrogen sulfite, make up to 100 mL with MeOH, inject a 10 μL aliquot. (IS solution was 250 mg methionine in 40 mL 0.05% sodium hydrogen sulfite, adjusted to pH 3 with phosphoric acid, sonicate, make up to 50 mL with MeOH.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak ODS
Mobile phase: MeCN:MeOH:buffer 0.8:0.6:98.6 (Buffer was 5 mM sodium hexanesulfonate adjusted to pH 2.9 with phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: 6.8
Internal standard: methionine (11)

OTHER SUBSTANCES
Simultaneous: S-carboxymethylcysteine, methylcysteine

KEY WORDS
tablets; capsules; granules

REFERENCE
Tsai,F.Y.; Chen,C.J.; Chien,C.S. Determination of the cysteine derivatives N-acetylcysteine, S-carboxymethylcysteine and methylcysteine in pharmaceuticals by high-performance liquid chromatography, *J.Chromatogr.A*, **1995**, *697*, 309–315.

SAMPLE
Matrix: formulations

Sample preparation: Dissolve solutions, powders, or powdered tablets in water to give a 200-325 μM solution, filter if necessary. Mix 500 μL solution with 500 μL 170 mM pH 7.5 borate buffer and 500 μL 2 mM 1,1-bis(phenylsulfonyl)ethylene (1,1'-ethenylidene-bis(sulfonyl)bis-benzene; Fluka, Merck) in MeOH, let stand at room temperature for 2 min, add 500 μL water, add 300 μL chloroform, vortex for 1 min, centrifuge for 2 min. Remove a 1 mL aliquot of the aqueous layer and add it to 500 μL 300 mM orthophosphoric acid, add 100-200 μL 30 μM IS in MeCN, mix, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:buffer 36:64 (Buffer was 50 mM pH 4.0 triethylamine-phosphate buffer.)
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 12
Internal standard: methyl p-hydroxybenzoate (10)
Limit of detection: 100 pmole

KEY WORDS

derivatization; tablets; powders

REFERENCE

Cavrini,V.; Gotti,R.; Andrisano,V.; Gatti,R. 1,1'-[Ethylidenebis(sulfonyl)]bis-benzene: A useful pre-chromatographic derivatization reagent for HPLC analyses of thiol drugs, *Chromatographia*, **1996**, *42*, 515–520.

SAMPLE

Matrix: solutions
Sample preparation: Mix 330 μL 3 mM 4-chloro-7-sulphobenzofurazan in 100 mM pH 9.2 borate buffer with 100 μL 1 M NaOH and 230 μL water containing excess of thiol (13.3 fold), after the appearance of a yellow color add 100 μL 1 M HCl, dilute to 1330 μL with 200 mM pH 7 phosphate buffer, inject an aliquot.

HPLC VARIABLES

Column: 150 × 3.9 3 μm Nova-Pak C18
Mobile phase: MeCN:150 mM H_3PO_4 5:95 adjusted to pH 2.5
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 510

CHROMATOGRAM

Retention time: 8.01

OTHER SUBSTANCES

Simultaneous: cysteinylglycine, reduced glutathione, cysteine

REFERENCE

Chen,X.; Cross,R.F.; Clark,; Baker,W.L. Chromatographic separation of fluorescent thiol adducts of 4-chloro-7-sulphobenzofurazan. Use as substrates for enzymes of the mercapturic acid xenobiotic pathway, *J.Chromatogr.B*, **1998**, *709*, 19–25.

SAMPLE

Matrix: solutions

Sample preparation: Add 1 mL 50 μg/mL N-(4-anilinophenyl)maleimide in 33 mM pH 6.85 phosphate buffer to 0.1-4 μg thiol, let stand at 0° for 90 min, wash twice with 2 mL portions of ether, heat the aqueous phase to 50° for 20 min, inject an aliquot. (Prepare N-(4-anilinophenyl)maleimide as follows. Add dropwise 1.1 g maleic anhydride in 10 mL chloroform to 1 g N-phenylphenylenediamine (4-aminodiphenylamine) stirred in 10 mL chloroform at 0°, filter, dry to give N-(4-anilinophenyl)maleamic acid. Heat 100 mg N-(4-anilinophenyl)maleamic acid and 25 mg sodium acetate in 400 μL acetic anhydride on a water bath for 2 h, cool, pour into ice-water, filter, recrystallize from ethyl acetate/hexane to give N-(4-anilinophenyl)maleimide as yellow needles (mp 135-6°).)

HPLC VARIABLES

Column: 305 × 6.3 μBondapak C18
Mobile phase: MeCN:0.5% pH 3.0 $(NH_4)H_2PO_4$ 4:7
Flow rate: 1
Injection volume: 10
Detector: E, Yanagimoto model VMD-101, glassy carbon electrode +1.0 V, Ag/AgCl reference electrode

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES

Simultaneous: L-cysteine, glutathione, D-penicillamine

KEY WORDS

derivatization

REFERENCE

Shimada,K.; Tanaka,M.; Nambara,T. Sensitive derivatization reagents for thiol compounds in high-performance liquid chromatography with electrochemical detection, *Anal.Chim.Acta*, **1983**, *147*, 375–380.

SAMPLE

Matrix: solutions
Sample preparation: Mix 1 mL of a 2 μM solution of thiols in 100 mM pH 8.0 sodium borate buffer containing 2 mM disodium EDTA with 1 mL 1 mM ABD-F in 100 mM pH 8.0 sodium borate buffer, vortex, heat at 50° for 5 min, cool in ice, add 600 μL 100 mM HCl, inject a 10 μL aliquot. (Synthesis of ABD-F is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is

a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°). Add 1 g CBD-F dropwise to 100 mL 6% ammonium hydroxide, neutralize with 10% HCl, evaporate under reduced pressure, add 200 mL MeCN to the residue, filter. Evaporate the filtrate and chromatograph on a 300 × 20 column of 100-200 mesh silica with chloroform, collect the appropriate fractions and evaporate them to give ABD-F (4-(aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole) as white needles (mp 145-6°) after recrystallization from n-hexane/benzene (Caution! Benzene is a carcinogen!).

HPLC VARIABLES

Guard column: 20 × 3.9 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 8-10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 4.0 potassium biphthalate buffer 8:92
Flow rate: 1
Injection volume: 10
Detector: F ex 380 em 510

CHROMATOGRAM

Retention time: 10
Limit of detection: 1.9 pmole

OTHER SUBSTANCES

Extracted: cysteamine, cysteine, glutathione, homocysteine

KEY WORDS

derivatization

REFERENCE

Toyo'oka,T.; Imai,K. New fluorigenic reagent having halogenobenzofurazan structure for thiols: 4-(Aminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole, *Anal.Chem.*, **1984**, *56*, 2461–2464.

SAMPLE

Matrix: solutions

Sample preparation: Mix 400 μL 2.5 μg/mL acetylcysteine in buffer with 100 μL 55 μg/mL N-(ferrocenyl)maleimide in acetone, let stand at 0° for 30 min, wash with three 2 mL portions of diethyl ether:hexane 50:50, inject an aliquot of the aqueous layer. (Buffer was 67 mM pH 6.8 phosphate buffer containing 1 mM EDTA. Prepare N-(ferrocenyl)maleimide as follows. Stir 13 g ferrocene in 200 mL anhydrous THF at -30° under nitrogen, add 160 mL 1.3 M butyllithium in ether dropwise over 25 min, stir at 0° for 2 h, stir at room temperature for 4 h. Stir at -20° and add 10.3 g methoxylamine in 75 mL anhydrous ether dropwise over 30 min, allow to warm gradually to room temperature, stir for 4 h, slowly add 10% HCl with stirring until the pH of the aqueous layer is 2, discard the organic layer. Make the aqueous layer strongly basic with KOH, extract with ether, extract the ether layer with 2 M HCl. Make the aqueous layer basic with KOH, extract with ether, dry over anhydrous magnesium sulfate, evaporate to dryness, recrystallize from ether/petroleum ether to obtain ferrocenylamine (8%, mp 140-145°) (J. Org. Chem. 1959, 24, 1487). Dissolve 300 mg ferrocenylamine in the minimum amount of chloroform, add 150 mg maleic anhydride in a little chloroform. Collect the compound that crystallizes and heat 300 mg with 1.5 g acetic anhydride and 60 mg anhydrous sodium acetate at 50-60° for 3 h with the exclusion of moisture (Chem. Zvesti 1963, 17, 21), recrystallize the product from diethyl ether to give N-(ferrocenyl)maleimide as deep purple prisms (mp 151-152°).

HPLC VARIABLES

Column: 150 × 4.6 5 μm YMC-GEL C8 (Yamamura, Kyoto)
Mobile phase: MeCN:buffer 20:50 (Buffer was 0.32% Na_2HPO_4 adjusted to pH 5.0 with phosphoric acid.)

Flow rate: 1
Detector: E, Environmental Sciences Associates 5100A, 5011 porous graphite dual electrode analytical cell, upstream electrode +150 mV, downstream electrode -100 mV, 5020 guard cell +200 mV, palladium reference electrode

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: L-cysteine, glutathione

KEY WORDS
derivatization

REFERENCE
Shimada,K.; Oe,T.; Nambara,T. Sensitive ferrocene reagents for derivatization of thiol compounds in high-performance liquid chromatography with dual-electrode coulometric detection, *J.Chromatogr.*, **1987**, *419*, 17–25.

SAMPLE
Matrix: solutions
Sample preparation: 1 mL Solution + 300 μL reagent solution, let stand at room temperature for 20 min, add 500 μL 300 mM phosphoric acid solution, make up to 10 mL with water, inject a 50 μL aliquot. (Prepare the reagent solution by dissolving 3.5 mg methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate in 10 mL THF, make up to 25 mL with pH 7.5 borate buffer. Prepare methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as follows. Dissolve 5 g 6'-methoxy-2'-acetonaphthone in warm glacial acetic acid and add 2.5 g glyoxylic acid, reflux for 24 h, evaporate to dryness under reduced pressure. Take up the residue in chloroform and extract it three times with 5% sodium carbonate solution. Combine the aqueous layers and acidify them with concentrated HCl, collect the product by filtration, recrystallize from MeOH/water or acetic acid to give 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid (mp 167-9°) (Farmaco, Ed. Sci. 1982, 37, 171). Reflux 0.5 g 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoic acid, 2.5 mL MeOH, and 2-3 drops sulfuric acid in 25 mL anhydrous benzene (Caution! Benzene is a carcinogen!) for 1 h, add 20 mL water, wash the organic layer with 10 mL 5% sodium bicarbonate solution, wash the organic layer with 20 mL water. Dry the organic layer over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, purify by flash chromatography on silica gel using ethyl acetate:light petroleum (bp 40-70°) 40:60 to give methyl 4-(6-methoxynaphthalen-2-yl)-4-oxo-2-butenoate as a pale yellow compound (mp 116-120°).)

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb RP-8
Mobile phase: MeOH:50 mM pH 3.0 triethylammonium phosphate 53:47
Flow rate: 1
Injection volume: 50
Detector: F ex 310 em 450

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: cysteamine, cysteine, glutathione, homocysteine, mesna
Noninterfering: bacitracin, biotin, calcium pantothenate, cystine, glycine, magnesium oxide, neomycin, starch, threonine, vitamin E, pyridoxine, riboflavin phosphate

KEY WORDS
solutions

REFERENCE
Gatti,R.; Cavrini,V.; Roveri,P.; Pinzauti,S. High-performance liquid chromatographic determination of aliphatic thiols with aroylacrylic acids as fluorogenic precolumn derivatization reagents, *J.Chromatogr.*, **1990**, *507*, 451–458.

SAMPLE
Matrix: solutions
Sample preparation: Mix 250 μL of a solution in 50 mM pH 9.3 borate buffer containing 1 mM disodium EDTA with 250 μL 1 mM SAOX-Cl in MeCN, let stand in the dark at room temperature for 1 h, add 500 μL MeCN:1 M HCl 50:50, inject an aliquot. (Prepare SAOX-Cl as follows. Gently reflux 21 g benzoin and 45 g urethane (Caution! Urethane is a carcinogen!) in 300 mL DMF for 6 h, cool, pour into water, filter, recrystallize to give 4,5-diphenyl-2-oxazolone (mp 211°) (Ber. 1956, 89, 1749). Carefully add 60 mL dimethylsulfamoyl chloride (?) to 7.3 g 4,5-diphenyl-2-oxazolone at 0°, heat at 55-60° for 4 h, cool, add dropwise to 500 g ice-water, filter, wash the solid with 4 L water. Add 100 mL dry benzene (Caution! Benzene is a carcinogen!) to 2 g of the crude material (4,5-bis(p-N,N-dimethylaminosulfonylphenyl)-2-oxazolone) and evaporate to dryness to remove traces of moisture, suspend the residue in 30 mL phosphorus oxychloride, stir at 0°, add 610 μL triethylamine dropwise, heat at 100° for 7 h, remove excess phosphorus oxychloride using a rotary evaporator. Dissolve the residue in dichloromethane and wash with cold saturated sodium bicarbonate, dry the organic layer over anhydrous magnesium sulfate, evaporate to dryness, chromatograph on 100 g silica gel with dichloromethane:ethyl acetate 90:10 to give SAOX-Cl (2-chloro-4,5-bis(p-N,N-dimethylaminosulfonylphenyl)oxazole) as a white solid (mp 222-224°) (Analyst 1993, 118, 257).)

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-8 (Supelco)
Mobile phase: MeCN:100 mM phosphoric acid 35:65
Flow rate: 1
Detector: F ex 330 em 425

CHROMATOGRAM
Retention time: 22.5
Limit of detection: 1.5 fmole

OTHER SUBSTANCES
Simultaneous: captopril, cysteine, glutathione, homocysteine, 2-mercaptopropionylglycine

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Chokshi,H.P.; Givens,R.S.; Carlson,R.G.; Lunte,S.M.; Kuwana,T. Fluorescence and chemiluminescence detection of oxazole-labelled amines and thiols, *Biomed.Chromatogr.*, **1993**, *7*, 208–216.

SAMPLE
Matrix: solutions
Sample preparation: Mix 100 μL N-acetylcysteine solution, 100 μL reagent solution, and 700 μL pH 8.4 borate buffer, vortex for a few s, let stand at room temperature for 1 h, add an equal volume of the mobile phase, inject a 10 μL aliquot. (Prepare reagent, 2-(4-N-maleimidephenyl)-6-methylbenzothiazole, as follows. Recrystallize 2-(4-aminophenyl)-6-methylbenzothiazole from chloroform before use. Add 500 mg maleic anhydride in 2 mL chloroform dropwise to 1.2 g 2-(4-aminophenyl)-6-methylbenzothiazole in 10 mL DMF, stir at room temperature for 2 h, filter, wash with 30 mL chloroform, recrystallize from DMF to give 2-(4-N-phenylmaleamic acid)-6-methylbenzothiazole as yellow crystals (mp 242°). Reflux 2 g 2-(4-N-phenylmaleamic acid)-6-methylbenzothiazole, 100 mg anhydrous sodium acetate, and 25 mL acetic anhydride for 2 h, cool on ice, filter, wash the solid with

water. Neutralize the filtrate with cold 10% NaOH, extract with chloroform. Dry the organic layer over anhydrous magnesium sulfate and evaporate it to dryness under reduced pressure. Combine this product with the solid obtained earlier and recrystallize from isopropanol to give 2-(4-N-maleimidephenyl)-6-methylbenzothiazole as yellow needles (mp 254-6°). Prepare the reagent solution by dissolving 50 μmoles of this compound in 10 mL DMF and diluting 25-fold with MeCN.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:buffer 35:65, pH 4.5 (Buffer was 10 mM KH_2PO_4 containing 0.1% sodium hexanesulfonate.)
Flow rate: 1.5
Injection volume: 10
Detector: F ex 320 em 405

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: N-acetylpenicillamine, coenzyme A, cysteine, glutathione, homocysteine, penicillamine

KEY WORDS
derivatization

REFERENCE
Haj-Yehia,A.I.; Benet,L.Z. Determination of aliphatic thiols by fluorometric high-performance liquid chromatography after precolumn derivatization with 2-(4-N-maleimidophenyl)-6-methylbenzothiazole, *Pharm.Res.*, **1995**, *12*, 155–160.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 200 μM solution in buffer with three volumes of a 400 μM solution of 5,5'-dithio-(bis-2-nitrobenzoic acid) in buffer, let stand at room temperature for 30 min, inject a 75 μL aliquot. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)

HPLC VARIABLES
Column: 250 × 4.6 Hypersil ODS1
Mobile phase: Gradient. MeCN:buffer 0:100 for 20 min, to 17.5:82.5 over 40 min. (Buffer was 125 mM NaH_2PO_4 containing 154 mM NaCl, pH adjusted to 7.4 with NaOH.)
Flow rate: 0.25 for 20 min, to 1 over 40 min
Injection volume: 75
Detector: UV 357

CHROMATOGRAM
Retention time: 41

OTHER SUBSTANCES
Simultaneous: N-acetylpenicillamine, captopril, cysteine, glutathione, penicillamine, thiomalic acid

KEY WORDS
derivatization

REFERENCE
Russell,J.; McKeown,J.A.; Hensman,C.; Smith,W.E.; Reglinski,J. HPLC determination of biologically active thiols using pre-column derivatization with 5,5'-dithio-(bis-2-nitrobenzoic acid), *J.Pharm. Biomed.Anal.*, **1997**, *15*, 1757–1763.

SAMPLE
Matrix: tissue
Sample preparation: Freeze tissue in liquid nitrogen and pulverize. Homogenize 50-100 mg tissue in 1 mL MeCN:20 mM EDTA 30:70, centrifuge at 4° at 4000 g for 5 min, adjust to 1-2.5% w/v with pH 8.4 borate buffer, keep on ice. 100 μL Sample + 100 μL 0.25 mM reagent in MeCN:DMF 95:5 + 700 μL pH 8.4 borate buffer, vortex for a few s, let stand for 1 h at room temperature, dilute with an equal volume of mobile phase, inject a 10 μL aliquot. (Reagent was 2-(4-maleimidophenyl)-6-methoxybenzofuran, a partial synthesis is given in the paper.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere-ODS
Mobile phase: MeCN:buffer 35:65 adjusted to pH 4.5 (Buffer was 10 mM KH_2PO_4 containing 0.1% sodium hexanesulfonate.)
Flow rate: 1.5
Injection volume: 10
Detector: F ex 310 em 390

CHROMATOGRAM
Retention time: 8
Internal standard: N-acetylcysteine

OTHER SUBSTANCES
Extracted: glutathione, homomocysteine, penicillamine, acetylpenicillamine

KEY WORDS
rat; heart; lung; liver; kidney; testes; spleen; N-acetylcysteine is IS

REFERENCE
Haj-Yehia,A.I.; Benet,L.Z. 2-(4-N-Maleimidophenyl)-6-methoxybenzofuran: a superior derivatizing agent for fluorimetric determination of aliphatic thiols by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *666*, 45–53.

SAMPLE
Matrix: urine
Sample preparation: Add 10 μL 50 μm 2-mercaptoethanol and 100 μL 10% trichloroacetic acid containing 10 mM EDTA to 100 μL urine, centrifuge at 760 g at 4° for 10 min. Add 350 μL 1 M pH 10.5 potassium borate buffer, 100 μL 1% tri-n-butylphosphine in water, and 100 μL 0.3% ammonium 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulphonate in water to a 150 μL aliquot of the supernatant yielding a final pH of about 8.5. Incubate the mixture at 60° for 60 min, then put in an ice bath and add 50 μL 4 M HCl, inject a 10 μL aliquot of this solution.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmosil 5C-18AR (Nakarai Tesque, Japan)
Mobile phase: MeOH:75 mM pH 2.9 sodium citrate buffer 2:98
Flow rate: 1
Injection volume: 10
Detector: F ex 386 em 516

CHROMATOGRAM
Retention time: 27.5

Internal standard: 2-mercaptoethanol

OTHER SUBSTANCES

Simultaneous: cysteamine, cysteine, homocysteine, cysteinylglycine, γ-glutamylcysteine, glutathione, homocysteine

KEY WORDS

derivatization; mouse

REFERENCE

Oe,T.; Ohyagi,T.; Naganuma,A. Determination of γ-glutamylglutathione and other low-molecular-mass biological thiol compounds by isocratic high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.B*, **1998**, *708*, 285–289.

SAMPLE

Matrix: urine

Sample preparation: Mix 5 mL urine with 200 μL 130 mM disodium EDTA, adjust pH to 9.8-10.0 with 5 M ammonia, make up to 6 mL with water, add 1 mL thiopropyl-Sepharose 6-B suspension, shake mechanically for 30 min, acidify to pH 3.5-4.0 with 1 mL 4 M acetic acid, centrifuge, add a 3-5 mL aliquot of the supernatant (containing up to 2 μmoles thiol) to a 13 × 7 p-acetoxymercurianiline Sepharose 4-B column, wash with 2 mL water, elute with 3 mL 10 mM cysteine hydrochloride. Add the eluate to a 25 × 5 column of 100-200 mesh AG 50 W-X8 (hydrogen form, Bio-Rad), elute with 1 mL 10 mM HCl, collect all the effluent (Clin. Chim. Acta 1979, 95, 189), add 200 μL 180 mM disodium EDTA. Remove a 250 μL aliquot and neutralize it with 25 μL 100 mM NaOH, add 5 mL 50 mM pH 9.0 carbonate buffer containing 10 mM disodium EDTA, add 500 μL 500 μM N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide in acetone, heat at 37° for 20 h, dilute 1:5 with mobile phase, inject a 100 μL aliquot. (Thiopropyl-Sepharose 6-B suspension contains 20 μmoles thiol/mL. Before use convert to free thiol form with dithiothreitol according to the manufacturer's instructions. Prepare p-acetoxymercurianiline-Sepharose 4B as follows. Mix 100 g Sepharose 4B with an equal volume of water, for each 1 mL Sepharose add 100 mg cyanogen bromide in an equal volume of water, adjust pH to 11 with 4 M NaOH, maintain at pH 11 with 4 M NaOH (Proc. Natl. Acad. Sci. USA 1968, 61, 636), at the end of the reaction (about 8 min), wash with 1.5 L 100 mM pH 9.0 sodium bicarbonate. Suspend in 100 mL DMSO:water 10:90 at 0°, slowly add 1.3 g 4-aminophenylmercuric acetate in 20 mL DMSO, stir slowly at 0° for 20 h, warm to 30°, filter, resuspend in 130 mL DMSO:water 20:80 at 35° for 5 min, filter, repeat this procedure 4 times, pack in a column, slowly wash with DMSO:water 20:80 until no mercury appears in the effluent (about 500 mL), store as a slurry in DMSO:water 20:80 (Biochim. Biophys. Acta 1970, 200, 593).)

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-8

Mobile phase: MeOH:buffer 15:85 (Buffer was 2 mM sodium phosphate buffer containing 11.8 mM tetramethylammonium hydroxide, pH adjusted to 7.4 with 6 M HCl.)

Flow rate: 0.75

Injection volume: 100

Detector: F ex 400 em 480

CHROMATOGRAM

Retention time: 20

Limit of detection: 53 fmole

OTHER SUBSTANCES

Extracted: mercaptoacetate

KEY WORDS

derivatization; SPE

REFERENCE

Kågedal,B.; Källberg,M. Reversed-phase ion-pair high-performance liquid chromatography of mercaptoacetate and N-acetylcysteine after derivatization with N-(1-pyrene)maleimide and N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide, *J.Chromatogr.*, **1982**, *229*, 409–415.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + EDTA, adjust pH to 9.8-10.0, react with thiopropyl-Sepharose, acidify with acetic acid, centrifuge. Remove a portion of the supernatant containing no more than 2 μmoles of thiols and add it to p-acetoxymercurianiline-Sepharose 4-B (Biochim. Biophys. Acta 1970, 200, 593), wash, elute with cysteine. Pass eluate through a small cation-exchange column (AG 50 W) (Bio-Rad) to remove cysteine, elute this column with 4 mL 10 mM HCl. (See Clin. Chim. Acta 1979, 95, 189.) Neutralize 250 μL eluate with 25 μL 100 mM NaOH, add 5 mL 50 mM pH 9.0 carbonate buffer containing 10 mM disodium EDTA, add 500 μL 0.5 mM N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide in acetone, heat at 37° for 20 h, dilute 1:5 with mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrosorb RP-8

Mobile phase: MeOH:2 mM sodium phosphate 15:85, containing 10 mM tetramethylammonium hydroxide, pH adjusted to 7.4 with 6 M HCl

Flow rate: 0.75

Injection volume: 100

Detector: F ex 400 em 480

CHROMATOGRAM

Retention time: 20

Limit of detection: 53 fmole

OTHER SUBSTANCES

Extracted: mercaptoacetate

REFERENCE

Kågedal,B.; Källberg,M. Reversed-phase ion-pair high-performance liquid chromatography of mercaptoacetate and N-acetylcysteine after derivatization with N-(1-pyrene)maleimide and N-(7-dimethylamino-4-methyl-3-coumarinyl)maleimide, *J.Chromatogr.*, **1982**, *229*, 409–415.

SAMPLE

Matrix: urine

Sample preparation: 250 μL Urine + 10 μL 14.5 mg/mL dithioerythritol in water + 100 μL phosphate buffer. After 30 min remove a 250 μL aliquot and add it to 250 μL citrate buffer and 50 μL 1 mg/mL 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole in MeOH, after 20 min inject a 20 μL aliquot. (Phosphate buffer was 17.8 g $Na_2HPO_4.2H_2O$ in 200 mL water, pH adjusted to 8.3 with NaOH. Citrate buffer was 29.4 g sodium citrate and 744 mg EDTA in 200 mL water, pH adjusted to 8.3 with NaOH.)

HPLC VARIABLES

Column: 300 × 4.6 7 μm Nucleosil C18

Mobile phase: MeCN:0.5% Na_2HPO_4 30:70

Column temperature: 30

Flow rate: 1.6

Injection volume: 20

Detector: UV 470

CHROMATOGRAM

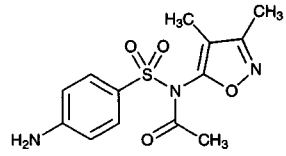
Retention time: 8

Limit of detection: 5 μM

KEY WORDS
derivatization

REFERENCE
Frank,H.; Thiel,D.; Langer,K. Determination of N-acetyl-L-cysteine in biological fluids, *J.Chromatogr.*, **1984**, *309*, 261–267.

Acetyl sulfisoxazole



Molecular formula: $C_{13}H_{15}N_3O_4S$
Molecular weight: 309.4
CAS Registry No.: 80-74-0
Merck Index: 9125

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 200 µL Plasma + 400 µL 0.2 µg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min. Remove the supernatant and evaporate it to 100 µL under a stream of nitrogen, inject a 50 µL aliquot. Urine. 100 µL Urine + 200 µL 12 µg/mL N^4-acetylsulfamethoxazole in MeOH, vortex for 10 s, centrifuge at 2000 rpm for 10 min, inject a 10 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 10 µm Lichrosorb RP-18 Hibar II
Mobile phase: MeOH:water:glacial acetic acid 32:68:0.06, pH adjusted to 4.7 with 4 M NaOH
Flow rate: 1.2
Injection volume: 10-50
Detector: UV 254

CHROMATOGRAM

Retention time: 9
Internal standard: N^4-acetylsulfamethoxazole (11.5)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: sulfisoxazole

KEY WORDS

plasma

REFERENCE

Jung,D.; Oie,S. "High-pressure" liquid chromatography of sulfisoxazole and N^4-acetylsulfisoxazole in body fluids, *Clin.Chem.*, **1980**, *26*, 51–54.

SAMPLE

Matrix: formulations
Sample preparation: Extract 1 mL Suspension with three 15 mL aliquots of chloroform, combine the organic layers and make up to 50 mL with chloroform, filter (0.45 µm silver membrane, Selas Corp.). Evaporate a 2 mL aliquot of the filtrate to dryness under a stream of nitrogen, reconstitute with 5 mL 330 µg/mL benzanilide in MeCN, inject a 5 µL aliquot.

HPLC VARIABLES

Column: 300 × 4 10 µm µBondapak C18
Mobile phase: MeCN:water 40:60
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 7

Internal standard: benzanilide (11)

OTHER SUBSTANCES

Simultaneous: sulfanilamide, sulfanilic acid, sulfisoxazole
Noninterfering: erythromycin ethylsuccinate

KEY WORDS

oral suspensions; suspensions

REFERENCE

Elrod,L.,Jr.; Cox,R.D.; Plasz,A.C. Analysis of oral suspensions containing sulfonamides in combination with erythromycin ethylsuccinate, *J.Pharm.Sci.*, **1982**, *71*, 161–166.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

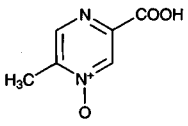
CHROMATOGRAM

Retention time: k' 2.86

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Acipimox



Molecular formula: $C_6H_6N_2O_3$
Molecular weight: 154.13
CAS Registry No.: 51037-30-0
Merck Index: 113

SAMPLE

Matrix: perfusate
Sample preparation: 1 mL Perfusate + 100 µL 20 µg/mL sulfanilamide + 1 mL 1 M phosphoric acid + 10 mL ethyl acetate:isopropanol 90:10, vortex for 3 min, centrifuge. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 150 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 4 µm Novapak phenyl in a Z-module radial compression module
Mobile phase: MeOH:buffer 4:96 (Buffer was 25 mM K_2HPO_4 + 5 mM tetrabutylammonium + 5 mM triethylamine, pH adjusted to 6.8 with concentrated phosphoric acid.)
Flow rate: 3
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Internal standard: sulfanilamide
Limit of quantitation: 100 ng/mL

REFERENCE

Ghabrial,H.; Czuba,M.A.; Stead,C.K.; Smallwood,R.A.; Morgan,D.J. Transfer of acipimox across the isolated perfused human placenta, *Placenta.*, **1991**, *12*, 653–661.

SAMPLE

Matrix: urine
Sample preparation: Dilute urine with water 1:10, inject a 20 µL aliquot directly.

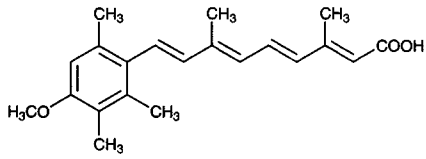
HPLC VARIABLES

Column: 250 × 3 Partisil PAC 10
Mobile phase: MeCN:pH 2.5 citrate-phosphate buffer 20:80
Flow rate: 1.4
Injection volume: 20
Detector: UV 269

REFERENCE

Musatti,L.; Maggi,E.; Moro,E.; Valzelli,G.; Tamassia,V. Bioavailability and pharmacokinetics in man of acipimox, a new antilipolytic and hypolipemic agent, *J.Int.Med.Res.*, **1981**, *9*, 381–386.

Acitretin



Molecular formula: $C_{21}H_{26}O_3$
Molecular weight: 326.44
CAS Registry No.: 55079-83-9
Merck Index: 114
Lednicer No.: 4 35

SAMPLE

Matrix: bile
Sample preparation: 20 µL Bile + 250 µL 1 M pH 5.5 acetate buffer + 20 µL Glusulase, shake at 37° for 4 h, inject a 100 µL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 Econosphere C18 (Alltech)
Mobile phase: MeOH:0.01% acetic acid 78:22
Flow rate: 1
Injection volume: 100
Detector: UV 280

CHROMATOGRAM

Retention time: 17.6

OTHER SUBSTANCES

Extracted: isoacitretin, Ro 23-4293, Ro 23-3571, metabolites

KEY WORDS

rat

REFERENCE

Cotler,S.; Chang,D.; Henderson,L.; Garland,W.; Town,C. The metabolism of acitretin and isoacitretin in the *in situ* isolated perfused rat liver, *Xenobiotica*, **1992**, *22*, 1229–1237.

SAMPLE

Matrix: bile, blood, perfusate, tissue
Sample preparation: Homogenize 1 g tissue and 4 mL ice-cold pH 7.4 Krebs-Henseleit buffer. Dilute bile with an equal volume of 200 mM pH 5 sodium acetate buffer. 100 µL Plasma, perfusate, diluted bile, or tissue homogenate + 20 µL MeCN + 350 µL MeCN:1-butanol 50:50 + 20 µL 37.6 µg/mL retinyl acetate, vortex for 1 min, add 300 µL 1 g/mL K_2HPO_4 in water, vortex for 30 s, centrifuge at 13600 g for 3 min, inject a 200 µL aliquot of the organic layer. (Hydrolyze conjugates in bile as follows. 100 µL Diluted bile + 8 µL 100000 U/mL β-glucuronidase (Helix pomatia, Sigma), heat at 37° for 5 h.)

HPLC VARIABLES

Guard column: 10 mm long Supelcosil LC-18 guard column
Column: 250 × 4.6 5 µm Supelcosil LC-18
Mobile phase: Gradient. A was MeCN:buffer 20:80. B was MeCN. A:B 65:35 for 10 min, 31:69 for 17 min (step gradient), re-equilibrate for 6 min. (Buffer was 0.8 g ammonium acetate and 10 mL glacial acetic acid in 200 mL water.)
Column temperature: 50
Flow rate: 1.5
Injection volume: 200
Detector: UV 350

CHROMATOGRAM

Retention time: 15.7 (acitretin), 15.4 (cis-acitretin)

Internal standard: retinyl acetate (25.0)
Limit of quantitation: 160 ng/mL

OTHER SUBSTANCES
Extracted: etretinate, metabolites

KEY WORDS
rat; liver; plasma; protect from light

REFERENCE
Decker,M.A.; Zimmerman,C.L. Simultaneous determination of etretinate, acitretin and their metabolites in perfusate, perfusate plasma, bile or hepatic tissue with reversed-phase high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *667*, 105–113.

SAMPLE
Matrix: blood
Sample preparation: 400 μL Plasma + 1.5 mL EtOH, freeze at -20° for 30 min, centrifuge. Inject a 1.4 mL aliquot of the supernatant onto column A and elute to waste with mobile phase A (time not given). Elute the contents of column A onto column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A C18-Corasil or C18-Lichrospher; B two 250 × 4 Supersphere 100 RP-18 end-capped columns in series
Mobile phase: A. MeCN:acetic acid (ratio not given) containing 1% ammonium acetate; B. Gradient. MeCN:acetic acid:10% ammonium acetate:water 60:1:6:30, 95:2:0.5:2, and 99:0.5:0:0.5 (times not given).
Detector: UV 360

CHROMATOGRAM
Internal standard: acitretin

OTHER SUBSTANCES
Extracted: isotretinoin, 4-oxo-isotretinoin, 4-oxo-tretinoin, tretinoin

KEY WORDS
plasma; column-switching; acitretin is IS

REFERENCE
Chen,C.; Mistry,G.; Jensen,B.; Heizmann,P.; Timm,U.; van Brummelen,P.; Rakhit,A.K. Pharmacokinetics of retinoids in women after meal consumption or vitamin A supplementation, *J.Clin.Pharmacol.*, **1996**, *36*, 799–808.

SAMPLE
Matrix: blood
Sample preparation: Mix 0.2-1 mL plasma and 100 μL buffer, extract with 2 mL diethyl ether:ethyl acetate 50:50 for 5 min. Centrifuge at 2000 g for 10 min at 4°, evaporate the organic phase to dryness. Dissolve the residue in 30-100 μL MeOH, inject an aliquot. (Solution was prepared in yellow amber glass and all handling was performed in a room with dim yellow light! Buffer was 25 mM KH_2PO_4 containing 40 mM Na_2HPO_4, pH 7.)

HPLC VARIABLES
Column: 250 × 4.6 4 μm Nova-Pak C18
Mobile phase: Gradient. A was MeCN:MeOH:THF 33.25:61.75:5. B was 2% acetic acid. A:B from 75:25 to 88:12 over 11 min, maintain at 88:12 for 19 min, return to initial condition at 30 min, equilibrate for 10 min.
Flow rate: 1

Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 21 (acitretin), 19.8 (13-cis-acitretin)
Internal standard: acitretin, 13-cis-acitretin

OTHER SUBSTANCES
Extracted: isotretinoin, tretinoin, 9-cis-retinoic acid
Noninterfering: acetaminophen, acyclovir, alprazolam, amikacin, amitriptyline, amphotericin B, aspirin, atenolol, bromazepam, caffeine, carbamazepine, ceftriaxone, chlorpromazine, cimetidine, clonazepam, dextromethorphan, diazepam, erythromycin, flunitrazepam, haloperidol, ketoconazole, lorazepam, meprobamate, metronidazole, methylprednisolone, miconazole, midazolam, nifedipine, nitrazepam, netilmicin, nordiazepam, nystatin, oxazepam, phenytoin, prednisolone, prednisone, sulconazole, theophylline, thiopental, zidovudine

KEY WORDS
plasma; rabbit; rat; acitretin is IS

REFERENCE
Disdier,B.; Bun,H.; Catalin,J.; Durand,A. Simultaneous determination of all-trans-, 13-cis, 9-cis-retinoic acid and their 4-oxometabolites in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *683*, 143–154.

SAMPLE
Matrix: blood
Sample preparation: 0.5-2 mL Plasma + 100 μL pH 7 phosphate buffer + 2 mL diethyl ether:ethyl acetate 50:50, vortex gently for 5 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 30-100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeOH:1% aqueous acetic acid 85:15
Flow rate: 1.5
Injection volume: 25
Detector: UV 350

CHROMATOGRAM
Retention time: 12
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: 13-cis-acitretin, tretinoin, etretinate, 4-oxo-13-cis-retinoic acid
Noninterfering: antidepressants, benzodiazepines, psoralen
Interfering: isotretinoin

KEY WORDS
plasma; handle under yellow light

REFERENCE
Bun,H.; al-Mallah,N.R.; Aubert,C.; Cano,J.P. High-performance liquid chromatography of aromatic retinoids and isotretinoin in biological fluids, *Methods Enzymol.*, **1990**, *189*, 167–172.

SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 10 μL 9.8 μg/mL retinyl palmitate in MeOH + 1.5 mL EtOH + 500 μL 2 M HCl, vortex for 30 s, add 5 mL water, vortex for 30 s, add 7.5 mL n-hexane, rotate for 15 min, centrifuge at 1500 g for 6 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm CP-Spher Si 5 μm (Chrompack)
Mobile phase: Dichloromethane:acetic acid 99.8:0.2 (?)
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 10 (13-cis), 11.5 (all-trans)
Internal standard: retinyl palmitate (6)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: etretinate

KEY WORDS
plasma; normal phase; pharmacokinetics; protect from light

REFERENCE
De Leenheer,A.P.; Lambert,W.E.; De Bersaques,J.P.; Kint,A.H. High-performance liquid chromatographic determination of etretinate and all-*trans*- and 13-*cis*-acitretin in human plasma, *J.Chromatogr.*, **1990**, *500*, 637–642.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 50 μL 1 μg/mL retinoic acid in methyl acetate + 500 μL pH 7.4 phosphate buffer + 200 μL methyl acetate + 4 mL diethyl ether, rotate at 20 rpm for 10 min, centrifuge at 2000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase. Inject a 150 μL aliquot.

HPLC VARIABLES
Column: 250 mm long 5 μm LiChrosorb Si 60
Mobile phase: Hexane:methyl benzoate:propionic acid 375:25:1
Flow rate: 2
Injection volume: 150
Detector: UV 365

CHROMATOGRAM
Retention time: 10.5
Internal standard: retinoic acid (5.2)
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: etretinate, isoacitretin

KEY WORDS
plasma; rat; normal phase; pharmacokinetics

REFERENCE
McNamara,P.J.; Blouin,R.A. Pharmacokinetic profile of two aromatic retinoids (etretinate and acitretin) in the obese Zucker rat, *J.Pharm.Sci.*, **1990**, *79*, 301–304.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 1 mL 100 ng/mL Ro 12-7554 and 100 ng/mL isotretinoin in EtOH, vortex, stand at 4° for 15 min, centrifuge at 1800 g for 3 min, inject a 500 μL aliquot onto column A with mobile phase A and elute for 7 min, elute column A in backflush mode with mobile phase A for 3 min, backflush contents of column A onto column B with mobile phase B and start the gradient for mobile phase B. At the end of the process flush the lines with component B of mobile phase B, re-equilibrate columns for 4 min. (Keep sample at 20° in the autosampler.)

HPLC VARIABLES

Column: A 14 × 4.6 37-50 μm Bondapak C18 Corasil (column fitted with 3 μm sieves not glass fiber filters); B 30 × 4 5 μm Spherisorb ODS 1 + 125 × 4 5 μm Spherisorb ODS 1

Mobile phase: A MeCN:1% ammonium acetate 10:90; B Gradient. A was MeCN:water:10% ammonium acetate:acetic acid 600:400:4:30. B was MeCN:water:10% ammonium acetate: acetic acid 850:146:4:10. A:B 100:0 to 0:100 over 8 min, stay at 0:100 for 11 min.

Flow rate: A 1.5; B 1

Injection volume: 500

Detector: UV 360

CHROMATOGRAM

Retention time: 16

Internal standard: Ro 12-7554 (ethyl all-trans-9-(2,6-dichloro-4-methoxy-m-tolyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate) (24) and isotretinoin (17)

Limit of detection: 0.5-1 ng/mL

Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES

Simultaneous: etretinate, 13-cis-acitretin, metabolites

KEY WORDS

plasma; column-switching

REFERENCE

Wyss,R. Determination of retinoids in plasma by high-performance liquid chromatography and automated column switching, *Methods Enzymol.*, **1990**, *189*, 146–155.

SAMPLE

Matrix: blood

Sample preparation: 500 μL Plasma + 10 μL 10 μg/mL 13-demethylretinoic acid in MeOH + 1.5 mL EtOH, vortex for 30 s, add 500 μL 2 M HCl, add 5 mL water, vortex for 30 s, add 7.5 mL n-hexane, rotate for 15 min, centrifuge at 1500 g for 6 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 150 μL mobile phase, inject a 50 μL aliquot. (Perform all manipulations in dim light.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Chromspher

Mobile phase: n-Hexane:methyl salicylate:acetic acid 200:18:0.6

Flow rate: 0.85

Injection volume: 50

Detector: UV 360

CHROMATOGRAM

Retention time: 12 (13-cis), 13 (all-trans)

Internal standard: 13-demethylretinoic acid (10)
Limit of quantitation: 3-4 ng/mL

KEY WORDS
plasma; normal phase

REFERENCE
Meyer,E.; Lambert,W.E.; De Leenheer,A.P.; Bersaques,J.P.; Kint,A.H. Improved quantitation of 13-*cis*- and all-*trans*-acitretin in human plasma by normal-phase high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *570*, 149–156.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma + 200 μL MeOH, vortex, add 200 μL 100 mM pH 7.4 phosphate buffer, add 5 mL 2.5 ng/mL retinoic acid in diethyl ether, rotate vertically at 20 rpm for 30 min, centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200 μL mobile phase, inject a 120 μL aliquot.

HPLC VARIABLES
Guard column: 30-40 μm pellicular C18 (Rainin)
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:1%acetic acid 83:17
Flow rate: 1
Injection volume: 120
Detector: UV 365

CHROMATOGRAM
Internal standard: retinoic acid
Limit of quantitation: 2 ng/mL

OTHER SUBSTANCES
Extracted: isoacitretin, vitamin A

KEY WORDS
plasma; rat; handle under yellow light; pharmacokinetics

REFERENCE
Small,D.S.; McNamara,P.J. Acitretin elimination in Sprague-Dawley rats pretreated with phenobarbital or β-naphthoflavone, *Drug Metab.Dispos.*, **1995**, *23*, 465–472.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg methyl-C1 Accubond SPE cartridge (J&W) with three 1 mL portions of MeOH and three 1 mL portions of 1% ammonium acetate. 500 μL Plasma + 20 μL MeCN containing 10 mM BHT + 1 mL 10 mM BHT in isopropanol, vortex, rotate for 15 min, centrifuge at 16000 g for 10 min. Remove the supernatant and add it to 11 mL 1% ammonium acetate, add to the SPE cartridge, wash with 1 mL 0.1% ammonium acetate, wash with 1 mL MeOH:0.1% ammonium acetate 50:50, dry under vacuum for 30 s, elute with 1.5 mL 10 mM BHT in MeCN. Add 10 μL pentafluorobenzyl bromide and 10 μL 10 mg/mL potassium carbonate in MeCN:water 50:50 to the eluate, vortex, let stand at room temperature for 1 h, evaporate to dryness under reduced pressure for 2 h, reconstitute with 20-100 μL 10 mM BHT in MeCN, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18 + 75 × 3.9 Nova-Pak C18 (in series)

Mobile phase: Gradient. MeCN:buffer 80:20 for 10 min, to 90:10 (step gradient). (Buffer was 100 mM ammonium acetate adjusted to pH 5.0 with acetic acid.)
Column temperature: 40
Injection volume: 20
Detector: UV 369, MS Hewlett-Packard model 5988A, particle beam interface nebulizer 60°, helium 35 psi, m/z 325

CHROMATOGRAM
Retention time: 16
Internal standard: acitretin

OTHER SUBSTANCES
Extracted: isotretinoin (m/z 299), tretinoin (m/z 299), 9-cis-retinoic acid (m/z 299)

KEY WORDS
plasma; protect from light; derivatization; SPE; acitretin is IS

REFERENCE
Lehman,P.A.; Franz,T.J. A sensitive high-pressure liquid chromatography/particle beam/mass spectrometry assay for the determination of *all-trans*-retinoic acid and 13-*cis*-retinoic acid in human plasma, *J.Pharm.Sci.*, **1996**, *85*, 287–290.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 300-1000 μL Plasma + 100 μL pH 7 Titrisol buffer + IS in MeOH + 2 mL diethyl ether:ethyl acetate 1:1, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 25°, reconstitute the residue in 30-50 μL MeOH, inject an aliquot. Tissue. Add IS, homogenize skin sample with 6 mL ethyl acetate:diethyl ether 1:1 with an 8 mm cutter at 24000 rpm below 21° (Ultra Turrax T25), centrifuge at 2000 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen below 21°, reconstitute the residue in 30-50 μL, inject an aliquot. (Protect all samples from light.)

HPLC VARIABLES
Column: 250 × 4.2 5 μm Nucleosil C18
Mobile phase: MeOH:MeCN:1.5% acetic acid 59.5:25.5:15
Flow rate: 1.2
Detector: UV 350

CHROMATOGRAM
Retention time: 6 (13-cis), 6.9 (all-trans)
Internal standard: arotinoid methyl sulfone (Ro 15-1570) (7.8)
Limit of quantitation: 1 ng/mL

KEY WORDS
plasma; skin

REFERENCE
Laugier,J.-P.; Surber,C.; Bun,H.; Geiger,J.-M.; Wilhelm,K.-P.; Durand,A.; Maibach,H.I. Determination of acitretin in the skin, in the suction blister, and in plasma of human volunteers after multiple oral dosing, *J.Pharm.Sci.*, **1994**, *83*, 623–628.

SAMPLE
Matrix: culture media
Sample preparation: 100 μL Culture media + 200 μL ice-cold EtOH, mix thoroughly, let stand for 15 min, centrifuge at 12000 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: Whatman CO:PELL ODS guard column
Column: 100 × 8 5 μm Nova-Pak C18 (radial-packed)
Mobile phase: MeOH:100 mM pH 7.0 ammonium acetate 90:10
Flow rate: 1
Detector: UV 340

CHROMATOGRAM
Retention time: 7.00
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: isotretin, motretinid, all-trans-retinoic acid, Vitamin A (retinol), retinal, etretinate

REFERENCE
Kochhar,D.M.; Penner,J.D.; Minutella,L.M. Biotransformation of etretinate and developmental toxicity of etretin and other aromatic retinoids in teratogenesis bioassays, *Drug Metab.Dispos.*, **1989**, *17*, 618–624.

SAMPLE
Matrix: perfusate
Sample preparation: 500 μL Perfusate + 1 mL acetone + 20 μL 26 μg/mL retinyl acetate, vortex for 1 min, centrifuge at 4° at 1300 g for 15 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: LC-18 pellicular (Supelco)
Column: 150 × 4.6 5 μm Supelcosil C18
Mobile phase: MeCN:water 84:16 containing 0.8 g/L ammonium acetate and 10 mL/L glacial acetic acid
Flow rate: 1.5
Detector: UV 350

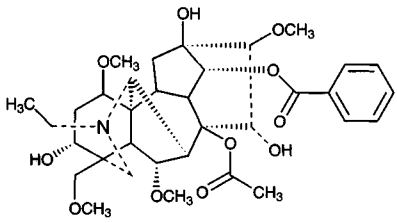
CHROMATOGRAM
Internal standard: retinyl acetate

OTHER SUBSTANCES
Extracted: etretinate

KEY WORDS
do not use PTFE or plastic; rat

REFERENCE
Pithavala,Y.K.; Odishaw,J.L.; Han,S.; Wiedmann,T.S.; Zimmerman,C.L. Retinoid absorption from simple and mixed micelles in the rat intestine, *J.Pharm.Sci.*, **1995**, *84*, 1360–1365.

Aconitine



Molecular formula: $C_{34}H_{47}NO_{11}$
Molecular weight: 645.75
CAS Registry No.: 302-27-2
Merck Index: 120

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform: isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 233

CHROMATOGRAM

Retention time: 5.95

Limit of detection: <120 ng/mL

KEY WORDS

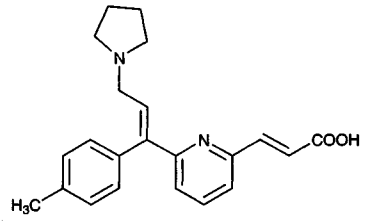
whole blood; plasma; interferences may occur—compounds(all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydra-

mine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

Acrivastine



Molecular formula: $C_{22}H_{24}N_2O_2$
Molecular weight: 348.44
CAS Registry No.: 87848-99-5
Merck Index: 129
Lednicer No.: 4 105

SAMPLE

Matrix: urine

Sample preparation: Condition a C18 SepPak SPE cartridge with 5 mL MeOH and 10 mL water. Urine. Centrifuge, add 0.1-7 mL to the SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen in a warm water bath, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject an aliquot. Feces. Lyophilize, grind to powder, weigh out 0.22-1.28 g, add 10 mL MeOH:water 80:20, vortex vigorously, sonicate using a microtip probe for 15 s, shake vigorously for 30 min, centrifuge, remove supernatant, repeat extraction (without sonication) twice. Combine extracts and dilute them with water to 20% MeOH concentration. Add to two SPE cartridges connected in series, wash with 3 mL water, elute with 4 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen in a warm water bath, reconstitute the residue in 300 μL MeOH, filter (0.45 μm), inject an aliquot. Plasma. 700 μL Plasma + 700 μL water, add to SPE cartridge, wash with 2 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness, reconstitute the residue in 200 μL MeOH, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES

Guard column: 5 μm C8 Spherisorb Octyl
Column: 150 × 4 5 μm Econosphere C8
Mobile phase: Gradient A. A was MeCN:100 mM ammonium acetate 5:95. B was MeCN: 100 mM ammonium acetate 50:50. A:B from 100:0 to 80:20 over 10 min then to 0:100 over 10 min, maintain at 0:100 for 5 min.
Flow rate: 0.9
Detector: UV 235

CHROMATOGRAM

Retention time: 15

OTHER SUBSTANCES

Extracted: metabolites

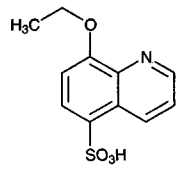
KEY WORDS

SPE; pharmacokinetics

REFERENCE

McNulty,M.J.; Deal,D.L.; Nelson,F.R.; Weller,S.; Chandrasurin,P.; Shockcor,J.; Findlay,J.W. Disposition of acrivastine in the male beagle dog, *Drug Metab.Dispos.*, **1992**, *20*, 679–687.

Actinoquinol



Molecular formula: $C_{11}H_{11}NO_4S$
Molecular weight: 253.28
CAS Registry No.: 15301-40-3, 7246-07-3 (sodium salt)
Merck Index: 143

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM

Retention time: 4.637

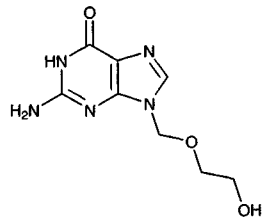
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Acyclovir



Molecular formula: $C_8H_{11}N_5O_3$
Molecular weight: 225.21
CAS Registry No.: 59277-89-3, 69657-51-8 (sodium salt)
Merck Index: 148
Lednicer No.: 3 229; 4 31; 4 116; 4 165

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 50 μL 35% perchloric acid, mix, centrifuge at 4° at 1500 g for 15 min. Inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Hypersil ODS
Mobile phase: 20 mM pH 3.5 KH_2PO_4
Flow rate: 1.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 9.8
Limit of detection: 2 ng
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Noninterfering: azathioprine, bumetamide, cyclosporine, cimetidine, dexamethasone, furosemide, ganciclovir, guanine, guanosine, hypoxanthine, 6-mercaptopurine, phenobarbital, prednisone, pristinamycine, pyostacine, ranitidine, salicylic acid, uric acid, vancomycine, xanthine

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Boulieu,R.; Gallant,C.; Silberstein,N. Determination of acyclovir in human plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *693*, 233–236.

SAMPLE

Matrix: blood
Sample preparation: 250 μL Plasma + 30 μL 60-62% perchloric acid, vortex for 30 s, centrifuge at 12800 g for 25 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 30-40 μm Perisorb RP-18 (Upchurch Scientific)
Column: 250 × 4 7 μm LiChrosorb RP-8
Mobile phase: MeCN:buffer 1:99 (Buffer was 20 mM NaH_2PO_4adjusted to pH 2.5 with 60–62% perchloric acid.)
Flow rate: 1.2
Injection volume: 50
Detector: F ex 270 em 380

CHROMATOGRAM

Retention time: 9.59

Limit of detection: 30 ng/mL
Limit of quantitation: 62.5 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Peh,K.K.; Yuen,K.H. Simple high-performance liquid chromatographic method for the determination of acyclovir in human plasma using fluorescence detection, *J.Chromatogr.B*, **1997**, *693*, 241–244.

SAMPLE
Matrix: blood
Sample preparation: Add 1 mL MeCN to 500 μL plasma, vortex briefly, centrifuge at 2000 g for 3 min, add 2 mL chloroform (Caution! Chloroform is a carcinogen!) to the supernatant, vortex. Remove the aqueous supernatant layer, remove traces of the organic solvent under a stream of nitrogen at 80° for 3 min, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrocart RP8
Mobile phase: MeCN:10 mM pH 5 ammonium acetate buffer 2:98
Flow rate: 1
Injection volume: 30
Detector: UV 254

CHROMATOGRAM
Retention time: 7
Internal standard: acyclovir (7)

OTHER SUBSTANCES
Extracted: gangiclovir

KEY WORDS
plasma; acyclovir is IS

REFERENCE
Cociglio,M.; Peyrière,H.; Hillaire-Buys,D.; Alric,R. Application of a standardized coextractive cleanup procedure to routine high-performance liquid chromatography assays of teicoplanin and ganciclovir in plasma, *J.Chromatogr.B*, **1998**, *705*, 79–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Sep-Pak Light C18 cartridge (Waters) with 1 mL MeOH and 1 mL water. Mix 500 μL serum with 500 μL 50% saturated NaCl in water or 100 μL urine with 900 μL 50% saturated NaCl in water. Push the mixture through the cartridge with a plastic syringe at a flow-rate 25 μL/s in this and subsequent steps, wash with 500 μL 50% saturated NaCl in water and elute with 1 mL 3% MeCN in 38 mM phosphoric acid. Collect the last 750 μL aliquot of the eluate and inject a 20 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:30 mM pH 2.1 phosphate buffer containing 5 mM dodecyl sulfate 18:82 (Prepare mobile phase as follows. Dissolve 4.08 g potassium dihydrogen phosphate, 1.45 g sodium dodecyl sulfate and 15 mL 3.85 mM phosphoric acid in 800 mL water, add 180 mL MeCN an make up to 1 L with water.)
Flow rate: 1.5
Injection volume: 20
Detector: F ex 285 em 380

CHROMATOGRAM
Retention time: 1.70
Limit of detection: 120 nM (plasma), 600 nM (urine)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: guanosine
Noninterfering: azathioprine, cyclosporine, furosemide, nifedipine, prednisolone, sulfamethoxazole, trimethoprim

KEY WORDS
SPE; serum; pharmacokinetics

REFERENCE
Svensson,J.-O.; Barkholt,L.; Säwe,J. Determination of acyclovir and its metabolite 9-carboxymethoxymethylguanine in serum and urine using solid-phase extraction and high-performance liquid chromatography, *J.Chromatogr.B*, **1997**, *690*, 363–366.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.073

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 10 μm CLC-ODS (Shimadzu)

Mobile phase: MeOH:2% acetic acid 10:90
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 13
Limit of detection: 1.22 μg/mL

OTHER SUBSTANCES
Simultaneous: guanine

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Zhang,S.S.; Liu,H.X.; Chen,Y.; Yuan,Z.B. Comparison of high performance capillary electrophoresis and liquid chromatography for the determination of acyclovir and guanine in pharmaceuticals and urine, *Biomed.Chromatogr.*, **1996**, *10*, 256–257.

SAMPLE
Matrix: tissue
Sample preparation: Extract skin (20 μm in thickness and 1.2 μL in volume) with 200 μL distilled water at 60° for 15 min, vortex twice for 10 s during extraction, cool, add 200 μL 148 mM perchloric acid, centrifuge at 5000 g for 10 min, filter (0.45 μm nylon, Lida, USA), inject an aliquot.

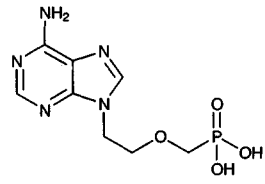
HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac C18
Mobile phase: Water
Flow rate: 1.2
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 9
Limit of quantitation: 8 ng/mL

KEY WORDS
skin

REFERENCE
Volpato,N.M.; Santi,P.; Laureri,C.; Colombo,P. Assay of acyclovir in human skin layers by high-performance liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *16*, 515–520.

Adefovir



Molecular formula: $C_8H_{12}N_5O_4P$
Molecular weight: 273.18
CAS Registry No.: 106941-25-7

SAMPLE

Matrix: blood
Sample preparation: Mix 100μL plasma with 200 μL 0.1% trifluoroacetic acid in MeCN. Evaporate to dryness under reduced pressure. Reconstitute dry sample in 200 μL derivatization solution, vortex, centrifuge. Remove the supernatant, incubate it at 95° for 40 min. Evaporate derivatized sample to dryness, reconstitute in 100 μL mobile phase A without MeCN. Inject a 50 μL aliquot. (Derivatization solution was 0.34% chloroacetaldehyde in 100 mM pH 4.5 sodium acetate.)

HPLC VARIABLES

Guard column: 15 × 3.2 Brownlee RP-18 Newguard
Column: 150 × 4.6 Zorbax RX-C18
Mobile phase: Gradient. A was MeCN:25 mM pH 6 potassium phosphate buffer containing 5 mM tetrabutyl ammonium hydrogen phosphate 2:98. B was MeCN:25 mM pH 6 potassium phosphate buffer containing 5 mM tetrabutyl ammonium hydrogen phosphate 65:45. A:B 100:0 for 2 min, to 0:100 over 13 min, return to 100:0 immediately.
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: F ex 236 em 420

KEY WORDS

plasma; derivatization; pharmacokinetics; dog

REFERENCE

Cundy,K.C.; Sue,I-L.; Visor,G.C.; Marshburn,J.; Nakamura,C.; Lee,W.A.; Shaw,J.-P. Oral formulations of adefovir dipivoxil: In vitro dissolution and in vivo bioavailability in dogs, *J.Pharm.Sci.*, **1997**, *86*, 1334–1338.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma, serum. Add trichloroacetic acid to 100 μL serum or plasma so that the final concentration of trichloroacetic acid is 100 mg/mL, shake vigorously for 10 min, centrifuge at 9000 g for 5 min, add the supernatant to an equal volume of tri-n-octylamine:Freon 20:80, shake vigorously for 30 min, discard the lower organic layer. Treat the aqueous layer with pH 4.7 ammonium acetate buffer (final concentration 160 mM) and chloroacetaldehyde (final concentration 40 mM), heat at 95° for 40 min, cool at 4°, inject an aliquot equivalent to 60 μL plasma or serum. Urine. Centrifuge urine, treat with pH 4.7 ammonium acetate buffer and chloroacetaldehyde, heat at 95° for 20 min (Antimicrob. Agents Chemother. 1996, 40, 22).

HPLC VARIABLES

Column: 125 × 4.6 4 μm Superspher 60 C8 (Merck)
Mobile phase: Gradient. A was MeCN:2.5 mM pH 5.0 $(NH_4)H_2PO_4$ containing 2 mM tetrabutylammonium hydrogen sulfate 5:95. B was MeCN:75 mM pH 5.0 $(NH_4)H_2PO_4$ containing 2 mM tetrabutylammonium hydrogen sulfate 15:85. A:B 100:0 for 4 min, to 0:100 over 2 min, maintain at 0:100 for 4 min, re-equilibrate at initial conditions for 8 min.
Flow rate: 1
Detector: F ex 254 (filter) em 425 (filter)

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CHROMATOGRAM

Retention time: 12.0
Limit of quantitation: 250 nM

OTHER SUBSTANCES

Extracted: adenosine monophosphate, (R,S)-9-(3-fluoro-2-phosphonylmethoxypropyl) adenine (FPMPA), (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (HPMPA)

KEY WORDS

monkey; cat; human; plasma; serum; derivatization

REFERENCE

Naesens,L.; Balzarini,J.; De Clercq,E. Acyclic adenine nucleoside phosphonates in plasma determined by high-performance liquid chromatography with fluorescence detection, *Clin.Chem.*, **1992**, *38*, 480–485.